

Investigation of Sectional Relationships in the Genus *Rhododendron* (Ericaceae) Based on *matK* Sequences

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Owing to the enormous number of species and conflicting ideas of classification proposed by many taxonomists, the genus *Rhododendron* poses systematic problems at various infrageneric levels. Sequence data derived from *matK* and *trnK* introns were used to examine relationships among all 8 subgenera and 12 sections of *Rhododendron*, and additional genera *Ledum*, *Menziesia*, and all *Elliotia*. The major results from this study are as follows: (1) *Menziesia* and *Ledum* are nested within the genus *Rhododendron*. (2) Thus *Rhododendron* is paraphyletic. (3) *R. camtschaticum* forms a basal lineage of tribe Rhodoreae. (4) Subgenus Tsutsusi is monophyletic; however, *R. tashiroi* makes both sections Tsutsusi and Brachycalyx para-/polyphyletic. (5) *R. tsusiophyllum* is a member of the subgenus Tsutsusi section Tsutsusi. (6) Subgenus *Rhododendron* is monophyletic. (7) Subgenus Pentanthera is polyphyletic. (8) Subgenus Pentanthera section Sciadorhodion is polyphyletic. (9) Subgenus Azaleastrum is polyphyletic.

In the mountain flora of East to Southeast Asia, the genus *Rhododendron* L. (Ericaceae) holds the essential position. The diversity of this group is represented by the immense number of species (e. g. over 1,000: Chamberlain et al. 1996) as well as variation in their habitat. Consequently, this group presents intricate taxonomic problems, in particular those on infrageneric circumscription.

Since Linnaeus (1753) established *Rhododendron*, taxonomists such as Don (1834), Planchon (1854), Maximowicz (1870), Wilson and Rehder (1921), and Copeland (1943) have attempted over the years to subdivide the ge-

nus in various ways. On the basis of these earlier works, Sleumer (1949, 1980) proposed a framework system of *Rhododendron* with a main emphasis upon relative positions of flower and leaf buds, characteristics of lepidote scales on the abaxial surface of leaves, and persistence or deciduousness of leaves as primary diagnostic characters. He recognized the following eight subgenera: Azaleastrum, Hymenanthus, Pentanthera, Pseudazalea, Pseudorhodorastrum, Rhododendron, Rhodorastrum, and Tsutsusi (Table 1).

Workers of the Royal Botanic Garden, Edinburgh, subsequently started to revise

Table 1. Comparison of two subdivisions of the genus *Rhododendron*

Sleumer (1980)			Chamberlain et al. (1996)		
Genus <i>Rhododendron</i>			Genus <i>Rhododendron</i>		
Subgen. <i>Rhododendron</i>	Sect. <i>Pogonanthum</i>	—————	Sect. <i>Pogonanthum</i>	Subgen. <i>Rhododendron</i>	
	Sect. <i>Rhododendron</i>	—————	Sect. <i>Rhododendron</i>		
	Sect. <i>Vireya</i>	—————	Sect. <i>Vireya</i>		
Subgen. <i>Pseudazalea</i>	Sect. <i>Pseudazalea</i>	—————			
Subgen. <i>Pseudorhodorastrum</i>	Sect. <i>Rhabdorhodon</i>	—————			
	Sect. <i>Rhodobotrys</i>	—————			
	Sect. <i>Trachyrhodon</i>	—————			
Subgen. <i>Rhodorastrum</i>	Sect. <i>Rhodorastrum</i>	—————			
Subgen. <i>Hymenanthus</i>	Sect. <i>Hymenanthus</i>	—————	Sect. <i>Ponticum</i>	Subgen. <i>Hymenanthus</i>	
Subgen. <i>Tsutsusi</i>	Sect. <i>Brachycalyx</i>	—————	Sect. <i>Brachycalyx</i>	Subgen. <i>Tsutsusi</i>	
	Sect. <i>Tsusiopsis</i>	—————			
	Sect. <i>Tsutsusi</i>	—————	Sect. <i>Tsutsusi</i>		
Subgen. <i>Pentanthera</i>	Sect. <i>Pentanthera</i>	—————	Sect. <i>Pentanthera</i>	Subgen. <i>Pentanthera</i>	
	Sect. <i>Rhodora</i>	—————	Sect. <i>Rhodora</i>		
	Sect. <i>Viscidula</i>	—————	Sect. <i>Sciadorhodon</i>		
		—————	Sect. <i>Viscidula</i>		
Subgen. <i>Azaleastrum</i>	Sect. <i>Azaleastrum</i>	—————	Sect. <i>Azaleastrum</i>	Subgen. <i>Azaleastrum</i>	
	Sect. <i>Choniastrum</i>	—————	Sect. <i>Choniastrum</i>		
	Sect. <i>Mumeazalea</i>	—————		Subgen. <i>Mumeazalea</i>	
	Sect. <i>Candidastrum</i>	—————		Subgen. <i>Candidastrum</i>	
Genus <i>Therorhodon</i>		—————		Subgen. <i>Therorhodon</i>	
Genus <i>Tsusiophyllum</i>		—————			

Sleumer's system. Cullen (1980) stressed the taxonomic importance of lepidote scales; he thus united all the lepidote (scaly) rhododendrons into a single subgenus *Rhododendron*, while Sleumer (1980) scattered them among four subgenera: *Rhododendron*, *Pseudazalea*, *Pseudorhodorastrum*, and *Rhodorastrum*. Philipson and Philipson (1986) assigned sections *Mumeazalea* and *Candidastrum* (of subgenus *Azaleastrum* sensu Sleumer) to a subgeneric rank, and they reduced genus *Therorhodon* to a subgeneric rank of the genus *Rhododendron*. Chamberlain and Rae

(1990) united the monotypic section *Tsusiopsis* sensu Sleumer with section *Tsutsusi*, and they also reduced the monotypic genus *Tsusiophyllum* to section *Tsutsusi*. Judd and Kron (1995) moved parts of sections *Brachycalyx* (of subgenus *Tsutsusi*) and *Rhodora* (of subgenus *Pentanthera*) to section *Sciadorhodon* (of subgenus *Pentanthera*). Chamberlain et al. (1996) compiled these results and recognized the following eight subgenera: *Azaleastrum*, *Candidastrum*, *Hymenanthus*, *Mumeazalea*, *Pentanthera*, *Rhododendron*, *Therorhodon*, and *Tsutsusi* (Ta-

ble 1). We will use this system as the reference, because (1) it covers the whole genus, and (2) it took account of the results from recent studies.

There are many studies that intended to clarify the phylogenetic relationships within *Rhododendron*, e.g., in terms of secondary substances (Harborne 1980, Harborne and Williams 1971, King 1980), interspecific cross compatibility (Williams et al. 1985, Yamaguchi et al. 1985), ontogeny (Palser et al. 1985, Philipson 1980), and micromorphology (Seith 1980). However, these results have been of limited value in determining the phylogeny of *Rhododendron*. Therefore, it is clear that we require another approach to resolve infrageneric systematic problems of *Rhododendron*.

Comparison of DNA sequences of *matK*, the maturase-encoding gene located in an intron of the chloroplast gene *trnK*, the former of which has evolved approximately three times faster than the chloroplast gene *rbcL* (Johnson and Soltis 1994), has proven to be a powerful tool for phylogenetic reconstruction within angiosperm families and genera (Johnson and Soltis 1994, Steele and Vilgalys 1994, Soltis et al. 1996). In this study, we compared *matK* and *trnK* introns sequences to investigate subgeneric and sectional relationships in *Rhododendron*.

Materials and Methods

Twenty-two species representing all eight subgenera and 12 sections of *Rhododendron*

recognized by Chamberlain et al. (1996) were sampled; since tribe Rhodoreae (of subfamily Rhododendroideae) comprises three genera, *Rhododendron*, *Ledum*, and *Menziesia*, we chose one species each from the latter two genera. A single species of *Elliottia* (subfamily Rhododendroideae tribe Cladothamneae) was also included. A single species of *Cassiope* (subfamily Vaccioideae tribe Cassiopeae) was selected as the outgroup based on the results of an analysis of *rbcL* (Kron and Chase 1993) and 18s rDNA (Kron 1996) sequences. Table 2 shows the materials examined and the sources where the plants were grown. All the voucher specimens are deposited in TNS.

Total DNA was extracted from fresh tissue following the methods of Kobayashi et al. (1995). Sequences were determined with PCR-amplified the *matK* gene and its flanking *trnK* introns from a total DNA extract by use of the primers shown in Fig. 1 and Table 3. Single-stranded DNA for dideoxy sequencing was produced in a second round of amplification using the double-stranded product as a template. Both the forward and reverse strands were sequenced for all taxa.

All parsimony analyses were conducted with PAUP, Phylogenetic Analysis Using Parsimony, Version 3.1 (Swofford 1993). The heuristic search option with 100 random replicates (Maddison 1991) was used to perform Fitch parsimony analyses (Fitch 1971). Branch lengths for trees were calculated by ACCTRAN optimization (Swofford and Maddison 1987).

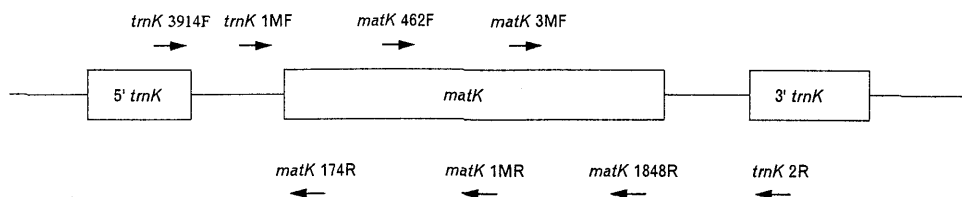


Fig. 1. Relative position of the PCR amplification and sequencing primers used for *matK* and *trnK* introns. Arrows indicate the direction of strand synthesis. Boxed areas represent coding regions.

Table 2. Species of *Rhododendron* and its related genera used for *matK* sequencing. Subdivision of *Rhododendron* is based on Chamberlain et al. (1996). Classification of all other taxa is based on Stevens (1971)

Species	Subgenus	Section	Voucher	GenBank accession
Subfamily Rhododendroideae				
Tribe Rhodoreae				
<i>Rhododendron ovatum</i> (Lindl.) Maxim.	Azaleastrum	Azaleastrum	Wilson 1391	AB012729
<i>Rhododendron stamineum</i> Franch.	Azaleastrum	Choniastrum	Akagi Nature Park 94/0084	AB012730
<i>Rhododendron albiflorum</i> Hook.	Candidastrum		Akagi Nature Park 95/0359	AB012731
<i>Rhododendron ponticum</i> L.	Hymenanthus	Ponticum	Apold, Cox & Hutchinson 205	AB012732
<i>Rhododendron semibarbatum</i> Maxim.	Mumeazalea		Kurashige 264	AB012733
<i>Rhododendron luteum</i> Sweet	Pentanthera	Pentanthera	Akagi Nature Park 89/0177	AB012734
<i>Rhododendron canadense</i> (L.) Torr.	Pentanthera	Rhodora	Akagi Nature Park 93/0150	AB012735
<i>Rhododendron schlippenbachii</i> Maxim.	Pentanthera	Sciadorhodion	Akagi Nature Park 92/0280	AB012736
<i>Rhododendron albrechtii</i> Maxim.	Pentanthera	Sciadorhodion	Kurashige 349	AB012737
<i>Rhododendron pentaphyllum</i> Maxim.	Pentanthera	Sciadorhodion	Kurashige 166	AB012738
<i>Rhododendron nipponicum</i> Matsum.	Pentanthera	Viscidula	Kurashige 241	AB012739
<i>Rhododendron primuliflorum</i> Bureau & Franch.	Rhododendron	Pogonanthum	SICH 143	AB012740
<i>Rhododendron ferrugineum</i> L.	Rhododendron	Rhododendron	Akagi Nature Park 90/0001	AB012741
<i>Rhododendron javanicum</i> (Blume) Benn.	Rhododendron	Vireya	Akagi Nature Park 86/0004	AB012742
<i>Rhododendron santapaui</i> Sastry & al.	Rhododendron	Vireya	Cox & Hutchinson 459	AB012743
<i>Rhododendron camtschaticum</i> Pall.	Therorhodion		Kurashige 458	AB012744
<i>Rhododendron farrerae</i> Tate apud Sweet	Tsutsusi	Brachycalyx	Akagi Nature Park 89/0021	AB012745
<i>Rhododendron wadanum</i> Makino	Tsutsusi	Brachycalyx	Kurashige 168	AB012746
<i>Rhododendron indicum</i> (L.) Sweet	Tsutsusi	Tsutsusi	Kurashige 195	AB012747
<i>Rhododendron kaempferi</i> Planch.	Tsutsusi	Tsutsusi	Ki 520-1	AB012748
<i>Rhododendron tashiroi</i> Maxim.	Tsutsusi	Tsutsusi	Kurashige 100	AB012749
<i>Rhododendron tsusiophyllum</i> Sugim.	Tsutsusi	Tsutsusi	Kurashige 771	AB012750
<i>Ledum palustre</i> L. subsp. <i>diversipilosum</i> (Nakai) H.Hara			Kurashige 476	AB012751
<i>Menziesia multiflora</i> Maxim.			Kurashige 356	AB012752
Tribe Cladothamneae				
<i>Elliottia paniculata</i> (Siebold & Zucc.) Benth. & Hook.			Kurashige 256C	AB012753
Subfamily Vaccinioideae				
Tribe Cassiopeae				
<i>Cassiope lycopodioides</i> (Pall.) D.Don			Kurashige 1124B	AB012754

For assessment of the relative robustness for clades found in each Fitch parsimony analysis, the bootstrap method (Felsenstein 1985) was used on 600 replicates.

Results

Our *matK* and its flanking *trnK* sequences provided a matrix of 2113 bp. A total of 106 nucleotide positions were phylogenetically informative in the matrix. Of the 20 indels identified from the aligned sequences, seven were informative and unambiguous. These indels were not used to construct the phylogenetic trees shown here, but the analysis combining the indel information also re-

sulted in the same topology. The phylogenetic analysis resulted in 84 most parsimonious trees each of 426 steps. These trees had a consistency index (CI) excluding uninformative characters of 0.695 and a retention index (RI) of 0.754. The strict consensus tree and one of the most parsimonious trees are shown in Figs. 2 and 3, respectively.

All of the most parsimonious trees supported the basal position of *Elliottia* (subfamily Rhododendroideae tribe Cladothamneae). The remaining genera in tribe Rhodoreae, *Ledum* and *Menziesia* were nested within genus *Rhododendron*. Consequently, the genus *Rhododendron* was shown to be

Table 3. Location and base composition of amplification and sequencing primers used for *matK* and *trnK* introns

Primer	5' sequence 3'								Designed by
<i>trnK</i> 3914F	GGG	GTT	GCT	AAC	TCA	ACG	G		Learn
<i>trnK</i> 1MF	GAT	AAG	TTT	ACC	GAG	GTA	GC		Yukawa
<i>matK</i> 462F	AAT	ACC	CTA	[C/T]CC		C[A/G]T		[C/T]CA TC	Chase
<i>matK</i> 3MF	GTG	GTC	TCA	ACC	AAG	AAG	G		Yukawa
<i>matK</i> 174R	CGA	[G/T]TA		ATT	AA[C/A]	CGT	TTC	AC	Chase
<i>matK</i> 1MR	GTA	GAA	AAA	ATC	GTA	ATA	GC		Yukawa
<i>matK</i> 1848R	TAT	CGA	ACT	TCT	TAA	TAG	C		Johnson/Soltis
<i>trnK</i> 2R	AAC	TAG	TCG	GAT	GGA	GTA	G		Steele

paraphyletic.

Rhododendron subgenus *Therorhodion* formed the basal lineage of tribe Rhodoreae with a bootstrap value of 99 %. In the core of tribe Rhodoreae, two major clades were apparent in all of the most parsimonious trees, but both clades are only weakly supported by bootstrap values. The first clade comprised *Rhododendron* subgenera *Candidastrum*, *Tsutsusi*, *Azaleastrum* (in part), *Pentanthra* (in part), and the genus *Menziesia* (Clade 1). Among the members of this clade, subgenus *Tsutsusi* formed a clade with a 99% bootstrap value. Within this clade, *R. tashiroi* (subgenus *Tsutsusi* section *Tsutsusi*) was nested within a clade made by the members of subgenus *Tsutsusi* section *Brachycalyx* (100 % bootstrap value). Although some taxonomists (e.g., Stevens 1971, Sleumer 1980, Yamazaki 1996) treated *R. tsusiophyllum* as a separate genus, *Tsusiophyllum*, this species was grouped with the members of subgenus *Tsutsusi* section *Tsutsusi* with a 99 % bootstrap value.

The second major clade consisted of the following taxa: *Rhododendron* subgenera *Pentanthra* (in part), *Rhododendron*, *Hymenanthes*, *Mumeazalea*, *Azaleastrum* (in part), and the genus *Ledum* (Clade 2). Among the members of this clade, a particularly well supported group was subgenus *Rhododendron*

(99 % bootstrap value). Within this clade, the sister group relationship of section *Rhododendron* to section *Pogonanthum* (bootstrap value of 93 %) as well as the monophyly of section *Vireya* (bootstrap value of 98 %) were suggested.

Subgenera *Pentanthra* and *Azaleastrum* were shown to be polyphyletic in this study. A part of subgenus *Pentanthra* section *Sciadorhodion* fell into Clade 1, but the rest of the same section formed a clade with the genus *Ledum* in Clade 2. *Rhododendron* subgenus *Pentanthra* sections *Pentanthra*, *Rhodora*, and *Viscidula* are also placed in Clade 2. The former two sections show a sister group relationship (59 % bootstrap value), and the latter formed a clade with the common ancestor of subgenus *Mumeazalea* and subgenus *Azaleastrum* section *Choniastrum* (58 % bootstrap value). Besides, subgenus *Azaleastrum* section *Choniastrum* was strongly grouped with subgenus *Mumeazalea* (bootstrap value 99 %) rather than with subgenus *Azaleastrum* section *Azaleastrum*, which was united with subgenus *Tsutsusi* in this study.

Discussion

Generic relationships in tribe Rhodoreae

The results of this study strongly suggest the paraphyly of *Rhododendron*, because gen-

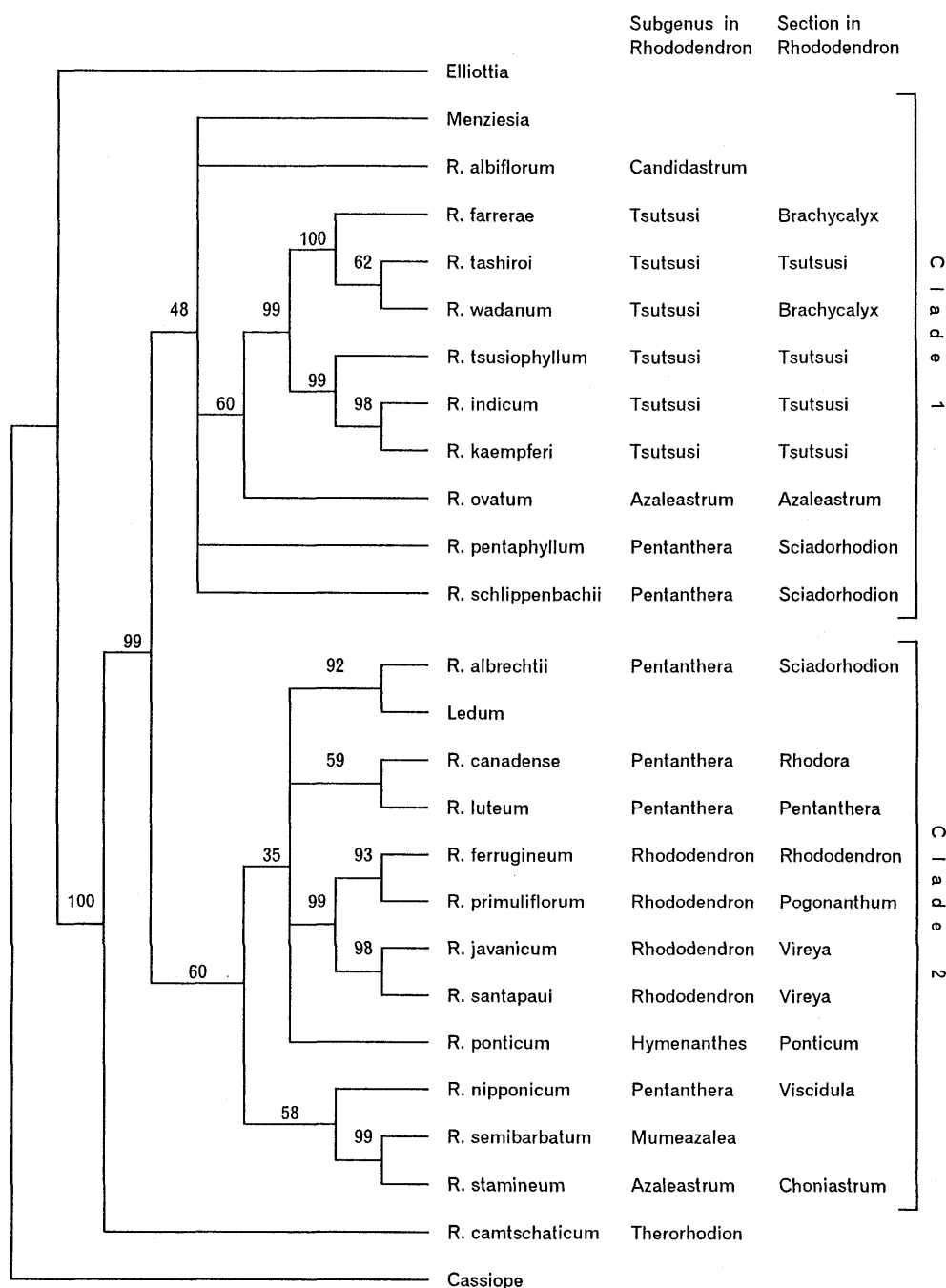


Fig. 2. Strict consensus of 84 most parsimonious Fitch trees based on *matK* and *trnK* intron sequences, length = 426; consistency index (excluding uninformative characters) = 0.695; retention index = 0.754. Bootstrap values from 600 replicates are provided above clades.

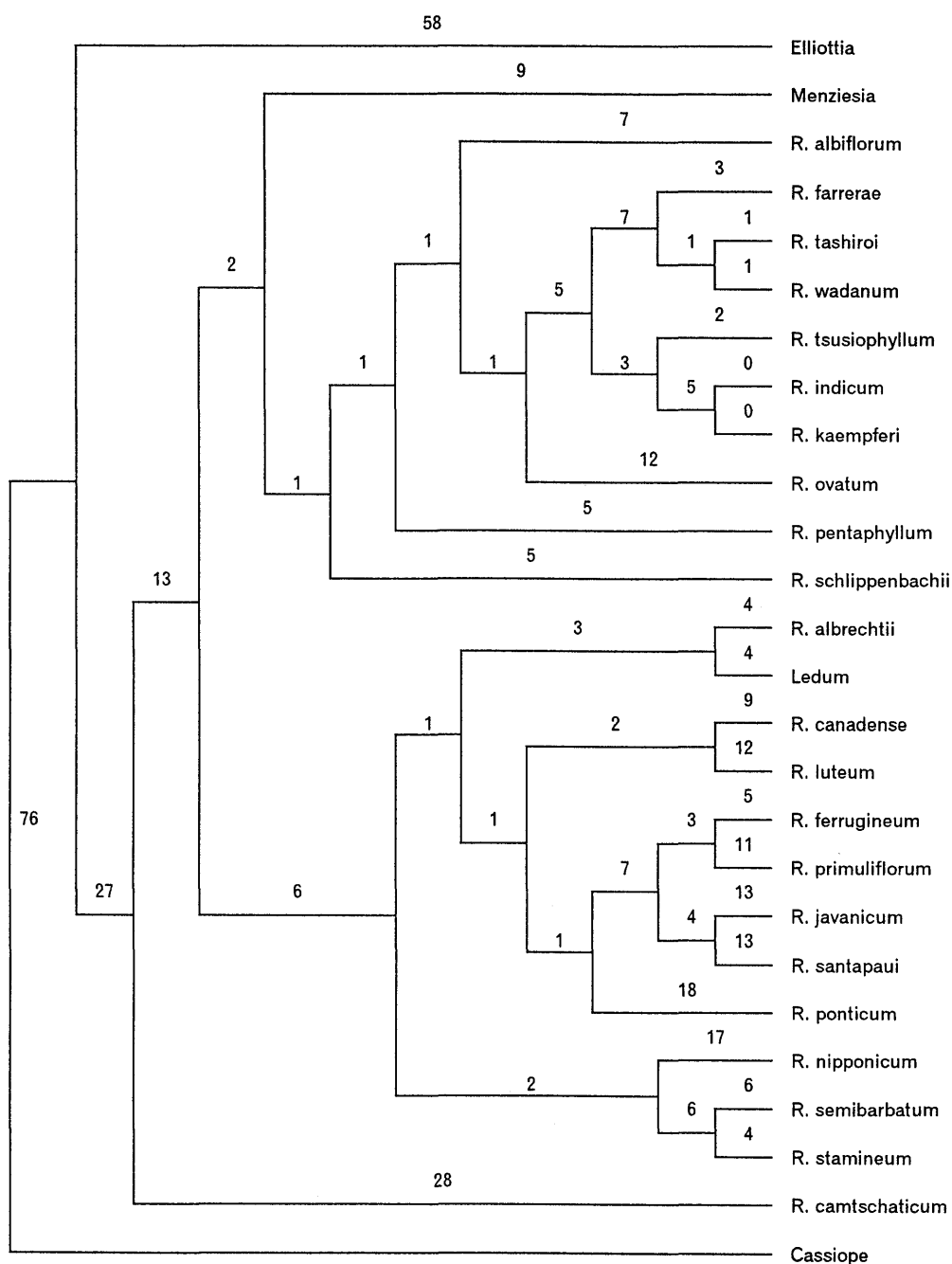


Fig. 3. One of 84 most parsimonious Fitch trees based on *matK* and *trnK* intron sequences. Number of nucleotide substitutions supporting each monophyletic group is indicated (ACCTRAN optimization).

era *Ledum* and *Menziesia* fall within the members of *Rhododendron*. However, if *Ledum* and *Menziesia* are included into *Rhododendron*, this genus becomes monophyletic. The paraphyly of *Rhododendron* is also suggested by a cladistic study based on morphological characters (Kron and Judd 1990), while placement of *Ledum* and *Menziesia* by Kron and Judd (1990) is different from ours.

Our results suggest that *Rhododendron* subgenus *Therorhodon* forms the basal lineage of tribe Rhodoreae (99 % bootstrap value). The following characters may warrant a separate generic status of subgenus *Therorhodon*: (1) results of this study; (2) chromosome numbers: $n=12$ in subgenus *Therorhodon*, $n=13$ in all other members of tribe Rhodoreae; (3) an elongated inflorescence axis with leaf-like bracts and bracteoles, a unique combination of characters in tribe Rhodoreae. A cladistic analysis using morphological characters of tribe Rhodoreae (Kron and Judd 1990) also supports the basal position of *Rhododendron* subgenus *Therorhodon*.

The genus *Ledum* fell within *Rhododendron* and formed a clade with *R. albrechtii* (subgenus *Pentanthera* section *Sciadorhodon*) with a 92 % bootstrap value. However, no anatomical or morphological features uniting these two taxa have been found yet. On the other hand, Kron and Judd (1990) reduced the genus *Ledum* to a subsectional rank of section *Rhododendron* based on the following synapomorphies: complex, multicellular, glandular, peltate scales; non-lacerate scales and simple trichomes fringing bud scales; and revolute leaves in leaf buds. However, our *matK* analysis does not reveal the close relationship of *Ledum* to section *Rhododendron*. Although the exact placement of *Ledum* in *Rhododendron* is uncertain, we are convinced that *Ledum* should be treated as an infrageneric taxon of the genus *Rhododendron*.

Menziesia has been widely recognized at a

generic rank (e.g., Stevens 1971, Sleumer 1980, Yamazaki 1989). Stevens (1971), however, suggested the close relationship of *Menziesia* to *R. tsusiophyllum* (genus *Tsusiophyllum* sensu Stevens) as both have anthers dehiscing through vertical short slits. By contrast, the remaining members of tribe Rhodoreae have anthers dehiscing by ovate pores. Kron and Judd (1990), in their cladistic analysis of morphological characters, also agreed with Stevens' (1971) placement of *Menziesia* with *R. tsusiophyllum*. Our results suggest that *Menziesia* falls among the members of *Rhododendron*, whereas *R. tsusiophyllum* does not form a clade with *Menziesia*. It is thus likely that a similar type of anther dehiscence in *Menziesia* and *R. tsusiophyllum* evolved independently. Further studies are required to assess the accurate phylogenetic position of *Menziesia* and the evolution of anther dehiscence.

Infrageneric relationships in *Rhododendron*

(1) Placement of *R. tashiroi* and *R. tsusiophyllum*

The monophyly of subgenus *Tsutsusi* was strongly supported by a 99 % bootstrap value. The position of *R. tashiroi*, however, makes both sections *Tsutsusi* and *Brachycalyx* para-/polyphyletic. Based on morphological information, the taxonomic position of *R. tashiroi* has not been stabilized. Having monomorphic leaves is a diagnostic character of section *Brachycalyx*, and having persistent leaves is that of section *Tsutsusi*. Sleumer (1980) thus recognized *R. tashiroi* as an intermediate taxon between sections *Tsutsusi* and *Brachycalyx*, and established a monotypic section *Tsusiopsis* (of subgenus *Tsutsusi*). On the other hand, Chamberlain and Rae (1990) moved this species to section *Tsutsusi*, because they gave emphasis to the taxonomic importance of its persistent leaves and flattened hairs on the

young stems. On the contrary, Yamazaki (1996) united this species with the members of section *Brachycalyx* based on its three pseudowhorled leaves at the shoot apex and filiform fringed hairs on both the leaves and stems. He designated this group section *Sciadorhodion* (the definition differs from that of Chamberlain et al. 1996). Our results strongly support Yamazaki's (1996) view, and allow the conclusion that both deciduous and persistent leaved species exist in section *Brachycalyx*.

Rhododendron tsusiophyllum was strongly grouped with all other species of subgenus *Tsutsusi* section *Tsutsusi* (99 % bootstrap value). Although some taxonomists (e.g., Stevens 1971, Sleumer 1980, Yamazaki 1996) treated this species as a separate genus, *Tsusiophyllum*, with emphasis on its vertical dehiscent anther and a three-loculated ovary, our results support the placement of this species into section *Tsutsusi* proposed by Chamberlain and Rae (1990). *Rhododendron tsusiophyllum* and *Rhododendron* section *Tsutsusi* share the following characters: multicellular, flattened, and ferruginous hairs; flower and leaf buds enclosed with terminal bud scales. Therefore it seems best to infer that the three-locular ovary and the vertical dehiscent anther of *R. tsusiophyllum* are autapomorphies of this species.

(2) Monophyly of subgenus *Rhododendron*

All of the sections of subgenus *Rhododendron* formed a monophyletic group (99 % bootstrap value). Section *Vireya* showed a sister group relationship to the common ancestor of sections *Rhododendron* and *Pogonanthum*. The following morphological characters are known to be unique to section *Vireya*: seeds with appendages (longer than seed bodies) at both ends; capsule valves twisted at the time of dehiscence; placentas separating as thread-like structures from the central axis, as well as the isolated distribution

mostly in Malesia. Our results showed a sister group relationship between sections *Rhododendron* and *Pogonanthum*. This close relationship is also supported by the following synapomorphies: entire scales on the abaxial surface of leaves, crenulate or undulate corollae, and simple trichomes fringing the flower bud scales.

(3) Polyphyly of subgenus *Pentanthera*

Subgenus *Pentanthera* is characterized by its deciduous leaves with indumentums of multicellular gland-headed and/or glandular hairs and unicellular hairs; terminal racemes and leafy shoots usually from axillary buds; and lack of gossypetin (Judd and Kron 1995). However, our results showed the polyphyly of subgenus *Pentanthera*: sections *Rhodora* and *Pentanthera* were shown to be sister groups in Clade 2; section *Sciadorhodion* was divided into Clades 1 and 2; the monotypic section *Viscidula* formed a clade with the common ancestor of subgenus *Mumeazalea* and subgenus *Azaleastrum* section *Choniastrum* in Clade 2. Several morphological characters also suggest the para-/polyphyly of this subgenus. Especially, the composition of leaf and flower buds of section *Sciadorhodion* is not uniform: in *R. schlippenbachii* both leaf and flower buds are enclosed with terminal bud scales, namely, the condition of a mixed bud; on the other hand, *R. albrechtii* and *R. pentaphyllum* develop an inflorescence from the terminal bud and a vegetative shoot from the lateral buds. Since the mixed bud is a diagnostic character of subgenus *Tsutsusi*, Sleumer (1980) submerged *R. schlippenbachii* into subgenus *Tsutsusi* section *Brachycalyx*. On the other hand, he included *R. albrechtii* and *R. pentaphyllum* into subgenus *Pentanthera* section *Rhodora* because the number of stamens and the corolla shape of these taxa are identical. Judd and Kron (1995) moved these three species to subgenus *Pentanthera* section *Sciadorhodion* on the basis of the following

synapomorphies: lack of gossypetin, deciduous leaves, and lack of stiff, ferrugineous, flattened, scale-like hairs on the abaxial surface of leaves. However, the strict consensus tree of this study is inconsistent with the recognition of Sleumer (1980) and Judd and Kron (1995). For elucidation of the relationships in section Sciadorhodon, accumulation of more phylogenetic signals from both molecular and conventional data as well as inclusion of *R. quinquefolium*, another member of this section, are required. At this moment, our best resolution suggests that the mixed bud evolved independently in *R. schlippenbachii* and subgenus Tsutsusi.

(4) *Relationships in the lateral-flowered group, subgenera Azaleastrum, Mumeazalea, and Candidastrum*

The monophyly of subgenus Azaleastrum was not supported in this study, because section Azaleastrum was grouped with subgenus Tsutsusi in Clade 1, and section Choniastrum showed a sister group relationship to subgenus Mumeazalea in Clade 2. Since Sleumer (1949, 1980) emphasized the taxonomic importance of the inflorescence from axillary buds, he thus recognized these species within a single subgenus, namely, Azaleastrum, and subdivided this subgenus into four sections Azaleastrum, Choniastrum, Mumeazalea, and Candidastrum on the basis of the number of stamens and deciduousness or persistence of leaves. However, our results do not support the monophyly of subgenus Azaleastrum sensu Sleumer. The close relationship of subgenus Azaleastrum section Choniastrum with subgenus Mumeazalea rather than with the remaining lateral-flowered taxa is inconsistent with results of previous studies based on morphology and geographic distribution. For example, subgenus Mumeazalea, a monotypic group endemic to Japan, has deciduous leaves, rotate corollae, five dimorphic stamens, and globose capsules; in contrast, section

Choniastrum is mainly distributed in southern China, and has persistent leaves, funnel-shaped corollae, ten stamens, and elongate capsules. Although the relationships among lateral-flowered taxa was not definitely elucidated in this study, it is probable that the character of lateral inflorescence evolved more than once in *Rhododendron*.

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約1,000種からなる大きな分類群, ツツジ属 (*Rhododendron*) は, 属内外の系統関係についてさまざまな見解がある。本研究では, Chamberlain et al. (1996) の分類体系を分子系統学的に検討する

ことを目的とし, この体系によるツツジ属の全亜属, 全節の少なくとも各1種を含む22種, さらにツツジ亜科のホツツジ属, イソツツジ属, ヨウラクツツジ属から各1種をサンプリングした。また外群としてスノキ亜科のイワヒゲ属を用いた。これ

らについて、葉緑体 DNA 上の *matK* 遺伝子と、その上流、および下流域の *trnK* イントロンをあわせて、2,113 bp の塩基配列マトリックスを比較した。解析の結果、以下の結果が示唆された。(1) イソツツジ属 (*Ledum*)、ヨウラクツツジ属 (*Menziesia*) は、ツツジ属に含まれる。(2) したがって、ツツジ属は偽系統群である。(3) エゾツツジ属として扱われることのあるエゾツツジ亜属 (Subgenus *Therorhodion*) は、ツツジ連で最初に分岐する系統である。(4) 独立属 (*Tsusiophyllum*) とされることのあるハコネコメツツジは、ツツジ属ヤマツツジ節 (subgenus *Tsutsusi* section *Tsutsusi*) に含まれる。(5) ツツジ亜属 (subgenus *Tsutsusi*) は単系統群であるものの、構成要素であるヤマツツジ節 (section

Tsutsusi) およびミツバツツジ節 (section *Brachycalyx*) は多/偽系統群である。サクラツツジ (*R. tashiroi*) をヤマツツジ節からミツバツツジ節に移すことで、両節は単系統になる。(6) ヒカゲツツジ亜属 (subgenus *Rhododendron*) は単系統群である。(7) レンゲツツジ亜属 (subgenus *Pentanthera*) は多系統群である。(8) レンゲツツジ属シロヤシオ節 (subgenus *Pentanthera* section *Sciadorhodion*) は多系統群である。(9) セイシカ亜属 (subgenus *Azaleastrum*) は多系統群である。

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